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(54) Title: COMPOSITION OF TYROSINE AND POLYMERISED ALLERGEN

(57) Abstract

A pharmaceutical composition comprising tyrosine and a polymerised allergen is prepared by (a) polymerising an allergen, (b) mixing an aqueous solution of the allergen with a solution of tyrosine in a strong aqueous acid, (c) neutralising the mixture of solutions, thereby co-precipitating tyrosine and polymerised allergen, and (d) optionally, mixing the product with a physiologically acceptable carrier.

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COMPOSITION OF TYROSINE AND POLYMERISED ALLERGEN

This invention relates to novel compositions for use in desensitisation therapy of allergy sufferers.

GB-A-1 492 973 describes a process for preparing coprecipitates of tyrosine having a modified allergen dispersed therein. The allergen has been modified by treatment with an agent, such as glutaraldehyde, which causes intra-molecular cross-linking and reduces the allergenicity of the product relative to the unmodified allergen.

EP-A-0 367 306 and Nakada et al (*Allergy, 54 [1985] 437 et seq.*) describe processes for preparing polymerised allergens by prolonged treatment with a cross-linking agent to cause intermolecular cross-linking, followed by filtration or dialysis to remove unpolymerised product. The polymerised allergens are described as having reduced allergenicity.

According to the present invention there is provided a pharmaceutical composition comprising tyrosine and a polymerised allergen. Typically, the allergen is coated with and /or adsorbed onto tyrosine, for example by co-precipitation.

The allergen may be derived from any allergy causing substance, such as a pollen (e.g. ragweed or birch pollen), food, insect venom, mould, animal fur, or house dust mite (*D. farinae* or *D. pteronyssinus*). In a particular aspect the allergen is derived from *D. pteronyssinus*. As used herein, "allergen" includes a mixture of allergens which may be from a single source or more than one source.

A further aspect of the invention provides a process for the preparation of a pharmaceutical composition in accordance with the invention, which process comprises (a) polymerising an allergen, (b) mixing an aqueous solution of the allergen with a solution of tyrosine in a strong aqueous acid, (c) neutralising the mixture of solutions, thereby co-precipitating tyrosine and polymerised allergen, and (d) optionally, mixing the product with a physiologically acceptable carrier.

Suitable physiologically acceptable carriers include phenol-saline and sterile water.

The allergen is polymerised by treatment with a dialdehyde such as glutaraldehyde, in aqueous solution at a pH of 3 to 10, typically 7±1 and a temperature of between 0 and 100 °C, typically between 4 and 37 °C, for up to 10 hours, for example about two hours at room temperature. The ratio of allergen to glutaraldehyde is typically in the range 1:25 to 1:2, for example about 1:4 w/w, although higher allergen ratios may be used in conjunction with a longer reaction time (see for instance EP-A-0 367 306, which uses ratios of about 3:1 and a reaction time of about seven hours).

Low molecular weight product is then removed by gel filtration or dialysis, for example, tangential flow dialysis, and the product freeze dried or used directly in the next stage. The molecular weight cut off is typically at least 100 kDaltons, for example at least 250 kDaltons, more preferably 300 kDaltons.

A solution of the polymerised allergen at pH 7±1, obtained either as the reaction mixture from the polymerisation process or from the solvation of a solid, is then mixed with a solution of tyrosine in a strong aqueous acid. The strong acid is usually an inorganic acid, preferably hydrochloric acid. The solution of polymerised allergen used in this step typically contains between 0.1 μg/ml and 100 μg/ml allergen protein. The ratio of allergen: tyrosine in the mixture is typically in the range $1:4 \times 10^5$ to $1:4 \times 10^2$ w/w.

The resulting mixture of solutions of allergen and tyrosine is neutralised. By neutralisation is meant an adjustment of pH to a value within the range 4.0 to 7.5. It is important that, at no time, or at least at no prolonged time, during the neutralisation does the pH of the solution rise appreciably above 7.5. This condition can be met by vigorous stirring of the solution and by the use only of the required amount of base, if desired. Various buffering agents can usefully be added to the solutions of allergen to assist in pH control during the mixing and neutralising stages.

A particularly useful method of carrying out the neutralisation is for separate streams of the solution of tyrosine in acid and the neutralising base to be run into the solution of allergen. The rates of flow of the added solutions are controlled by pH-state, that is by equipment which regulates the flow of one or both of the solutions so that the pH of the reaction mixture remains substantially constant at a predetermined level. We have found that optimum results are usually obtained by pH control within the range 6.5 to 7.5 though the precise pH may vary according to the nature of the allergen.

The result of the neutralisation is the immediate precipitation of the tyrosine, within and/or upon which the solution of allergen is occluded and/or adsorbed. After the precipitation the mixture is either washed immediately or allowed to stand for a period of from a few hours to a day or two prior to washing. Desirably the precipitate is obtained as fine as possible and this is achieved by rapid neutralisation of the solution coupled with vigorous agitation while this is being carried out.

The resulting precipitate may be removed from the solution by centrifugation or filtration and washed, e.g. with phenol-saline, before being resuspended in a physiologically-acceptable carrier such as phenol-saline, or sterile water, to produce an injectable composition suitable for use in desensitisation therapy.

The following Example illustrates the present invention:

Example

A neutral solution of approximately 2.5 mg/ml *D. pteronyssinus* extract protein which had been partially purified by dialysis or fractionation was polymerised by the addition of an equal volume of 1% w/v glutaraldehyde and the mixture stirred for approximately 2 hours at room temperature. The reaction was quenched by the addition of an equal volume of 2% w/v glycine and the mixture stirred for a further one hour at room temperature. Low molecular weight material was removed by diafiltration across a membrane with a molecular weight exclusion of 300 kDaltons. The mixture was then sterile filtered and freeze dried.

A solution of the polymerised allergen was prepared either directly from the sterile filtered solution or by reconstitution of the freeze dried solid. This solution contained 10µg/ml in phosphate buffer pH 7±1. The allergen solution was co-precipitated with tyrosine by the simultaneous addition of one volume of L-tyrosine in HCl (prepared by dissolving 24g L-tyrosine to 100ml with 3.4M HCl) and one volume of 3.2M NaOH, to four volumes of allergen solution, with vigorous agitation. The suspension so formed was centrifuged, washed repeatedly with buffered saline to remove contaminants and resuspended to the original volume in buffered saline pH6 ±1.

Claims

1. A pharmaceutical composition comprising tyrosine and a polymerised allergen.

2. A composition according to claim 1 wherein the allergen is coated with and/or adsorbed onto tyrosine.

3. A composition according to claim 1 or 2 wherein the allergen is derived from *D. pteronyssinus*.

4. A process for the preparation of a pharmaceutical composition according to any one of the preceding claims, which process comprises (a) polymerising an allergen, (b) mixing an aqueous solution of the allergen with a solution of tyrosine in a strong aqueous acid, (c) neutralising the mixture of solutions, thereby co-precipitating tyrosine and polymerised allergen, and (d) optionally, mixing the product with a physiologically acceptable carrier.

5. A composition according to any one of claims 1 to 3, for use in therapy.

6. A composition according to any one of claims 1 to 3, for use in desensitization therapy of allergy sufferers.

7. Use of a composition according to any one of claims 1 to 3 in the manufacture of a medicament for use in desensitization therapy of allergy sufferers.

INTERNATIONAL SEARCH REPORT

Ir ational Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/35 A61K39/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,4 070 455 (GREEN GEOFFREY ET AL) 24 January 1978 see the whole document ---	1-7
X	EP,A,0 058 021 (BEECHAM GROUP PLC) 18 August 1982 see the whole document ---	1-7
X	FR,A,2 145 555 (BEECHAM GROUP LTD) 23 February 1973 see the whole document ---	1,2,4-7
A	EP,A,0 367 306 (CBF CORP BIOLOG FARMA SA) 9 May 1990 cited in the application -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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